General-base catalysed hydrolysis and nucleophilic substitution of activated amides in aqueous solutions

Buurma, NJ; Blandamer, MJ; Engberts, JBFN; Buurma, Niklaas J.

Published in:
Journal of physical organic chemistry

DOI:
10.1002/poc.607

JOURNAL OF PHYSICAL ORGANIC CHEMISTRY
J. Phys. Org. Chem. 2003; **16:** 438–449
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/poc.607

General-base catalysed hydrolysis and nucleophilic substitution of activated amides in aqueous solutions†

Niklaas J. Buurma,[1] Michael J. Blandamer[2] and Jan B. F. N. Engberts[1]*

[1]Physical Organic Chemistry Unit, Stratingh Institute, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands
[2]Department of Chemistry, University of Leicester, Leicester LE1 7RH, UK

Received 7 October 2002; revised 30 October 2002; accepted 30 October 2002

ABSTRACT: The reactivity of 1-benzoyl-3-phenyl-1,2,4-triazole (**1a**) was studied in the presence of a range of weak bases in aqueous solution. A change in mechanism is observed from general-base catalysed hydrolysis to nucleophilic substitution and general-base catalysed nucleophilic substitution. A slight tendency is also observed for the more hydrophobic general bases to show higher reactivity towards **1a**. Aspartame is an effective nucleophile, possibly because nucleophilic substitution is subject to intramolecular general-base catalysis. A general conclusion derived from the present results is that unexpected rate effects can only be rationalised provided that the detailed reaction mechanisms are well understood. Copyright © 2003 John Wiley & Sons, Ltd.

KEYWORDS: hydrolysis; changes in mechanism; general-base catalysis; general-acid catalysis; nucleophilic substitution

INTRODUCTION

The hydrolysis reaction of the activated amide 1-benzoyl-3-phenyl-1,2,4-triazole (**1a**) is general-base catalysed (Scheme 1).[1]

In highly aqueous solutions, the concentration of water is sufficiently high for water to act (detectably) as both a general base and a nucleophile. Hence, in the absence of other general bases, the water-catalysed (i.e. pH-independent) hydrolysis is the sole reaction. In the presence of sufficiently basic cosolutes, the water-catalysed reaction is unimportant. More basic cosolutes are much more effective catalysts for hydrolysis than water. Consequently, despite the relatively low molality of added general bases, the general-base catalysed hydrolysis pathway competes with the water-catalysed pathway. It is stressed that even though in the water-catalysed reaction the second water molecule in the activated complex (i.e. B = H_2O, Scheme 1) acts as a general base, a distinction is drawn between the water-catalysed reaction and general-base catalysed reaction.

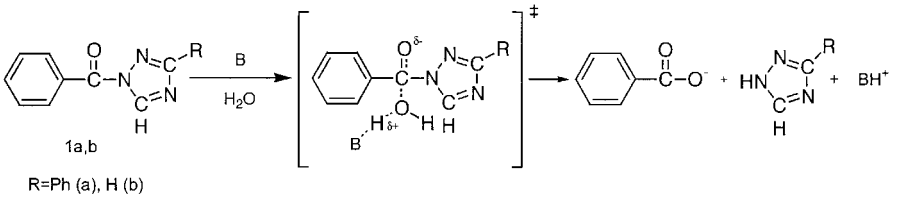
Increased basicity of cosolutes usually leads to a concomitant increase in nucleophilicity. This increase in nucleophilicity provides an alternative reaction pathway: nucleophilic attack on the carbonyl functionality, followed by loss of the (substituted) 1,2,4-triazole leaving group (Scheme 2).

In this mechanism, the nucleophilic water molecule is replaced by a stronger nucleophile. The similarity with the water-catalysed hydrolysis goes even further, as the nucleophilic substitution reaction can also be catalysed by general acids and bases. Some of the possible catalysed reaction pathways for nucleophilic substitution[2–4] are illustrated in Scheme 3, there are many different pathways for reactions of nucleophiles with amides and esters. The exact pathway depends on factors such as leaving group ability, nucleophilicity and solvent. Here, we shall only discuss those reactions pathways, that, we contend, are relevant for the system under study.

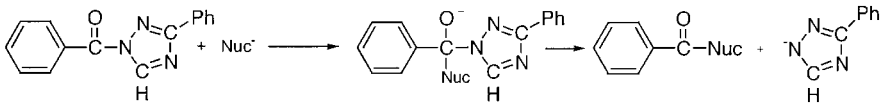
If nucleophilic attack is the rate-determining step, the reaction can be catalysed by a general base or by hydroxide, deprotonating the nucleophile. Similarly, the negative charge developing on the amide carbonyl can be stabilized by general acids, resulting in general-acid catalysis. However, if expulsion of the 3-phenyl-1,2,4-triazole from a protonated tetrahedral intermediate is rate determining, the reaction could be general-base catalysed. Further, departure of the leaving group may be general-acid catalysed.

In this intricate play of reactivity, reactants can assume many different roles, resulting in a series of related but different reaction pathways. It is therefore of paramount importance to have a detailed understanding of these reaction pathways if we are to fully understand the observed rate effects induced by general bases. We therefore studied the effect of a range of general bases on

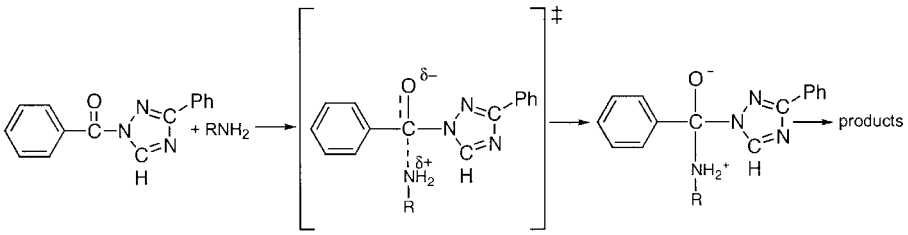
**Correspondence to:* J. B. F. N. Engberts, Physical Organic Chemistry Unit, Stratingh Institute, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands.
E-mail: j.b.f.n.engberts@chem.rug.nl
†Dedicated to Professor S. Kobayashi on the occasion of his retirement from academia.



Scheme 1



Scheme 2



Scheme 3

the reactivity of **1a** in order to identify possible competing reaction pathways. The results are used in a reinterpretation of the previously reported[5] rate-accelerating effects of some α-amino acids on the hydrolysis of **1a**.

We have previously shown that encounter complexes between reactive probes and added inert cosolutes can be stabilised by hydrophobic interactions.[6] However, if the cosolute is not inert, formation of an encounter complex constitutes the first step for bimolecular (or higher molecularity) reactions. Hence, if a cosolute reacts with the reactive probe, or catalyses the reaction of the reactive probe, more *hydrophobic* cosolutes are expected to show slightly higher reactivity than *hydrophilic* cosolutes with identical functional groups. Similarly, in a linear free energy relationship, more hydrophobic reactive cosolutes are expected to show deviations towards higher reactivity provided that hydrophobic interactions are not of similar importance in both processes.

The molecular picture of an unreactive cosolute blocking the reactive centre of the activated amide from attack by water proposed by us before[6–8] is expected to be equally valid in general-base catalysed hydrolysis and (catalysed) nucleophilic substitution. In buffer solutions of general bases in which the conjugate general acid is also present, intriguing compensating effects are possible. Increasing the hydrophobicity of the general base will lead to an increased efficiency in general-base catalysis (see above) and to a concomitant increase in the rate-retarding effect of the conjugate general acid.

RESULTS AND DISCUSSION

General-base catalysis by carboxylate ions and water

The effect was determined of different general bases on the (pseudo-)first-order rate constant of hydrolysis of **1a**. In all cases, at low molality and constant buffer ratio, the increase in rate constants was linear with increasing molality of added general base (Fig. 1).

In order to obtain the sole effect by the general bases, the effect of the conjugate general acid on the observed rate has to be calculated. In the absence of general base, the general acids were found to decrease the rate of the water-catalysed reaction. We attribute this decrease to blocking by the cosolute of the reaction centre from attack by water.[6–8] We contend that a similar inhibition of reaction occurs for the general-base catalysed hydrolysis.

Hypothetically, in the absence of inhibition by cosolute and in the pH-range in which the reaction without added general bases is only water-catalysed, the rate constant is described by the equation

$$k(m_b) = k(m_c = 0) + m_b k_b \qquad (1)$$

where m_b is the molality of general base, k_b is the (pseudo-)second-order rate constant for catalysis by the general base and $k(m_c = 0)$ is the (pseudo-)first-order rate constant in the absence of cosolute. Previously, Eqn.

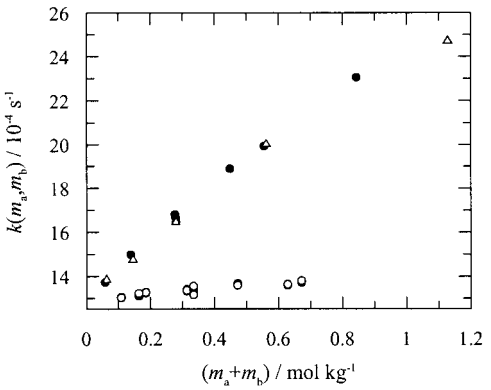


Figure 1. Effect of added ethanoate/ethanoic acid (△), butanoate/butanoic acid (●) and chloroethanoate/chloroethanoic acid (○) buffers (all buffer ratios 3:1 and pH values of 5.15 ± 0.10, 5.20 ± 0.10 and 3.20 ± 0.10, respectively; m_a and m_b are the molalities of acid and base, respectively) on the hydrolysis of **1a**

(2) has been employed to describe the effects of inert cosolutes on the water-catalysed hydrolysis reactions:

$$\ln\left[\frac{k(m_c)}{k(m_c=0)}\right] = \frac{2}{RTm_0^2}[g_{cx} - g_{c\neq}]m_c - N\phi M_1 m_c \quad (2)$$

where $k(m_c)$ is the (pseudo-)first-order rate constant in an m_c molal aqueous solution of inert cosolute c, $k(m_c = 0)$ the rate constant in the absence of added cosolute, R the gas constant and T the absolute temperature. Significantly, $[g_{cx} - g_{c\neq}]$ is the difference in interaction Gibbs energies between the cosolute c and the reactants x on the one hand and the activated complex ≠ on the other. M_1 is the molar mass of water, N is the number of water molecules involved in the rate-determining step and ϕ is the practical osmotic coefficient for the aqueous solution where the molality of added solute is m_c. For the water-catalysed hydrolysis, $N = 2$. Further, the solutions are very dilute and hence, ϕ can be taken as unity; m_0 is the (hypothetical) ideal reference state and corresponds to 1 mol kg^{-1}. The term $[g_{cx} - g_{c\neq}]$ is denoted as G(c). Equation (3) is a simpler form of Eqn. (2):

$$\ln\left[\frac{k(m_c)}{k(m_c=0)}\right] = am_c \quad (3)$$

where m_c is the molality of inert cosolute c and a quantifies the rate effect induced by cosolute c. Equation (3) can be rewritten as

$$k(m_c) = k(m_c = 0)e^{am_c} \quad (4)$$

The conjugate acid of the general-base catalyst is the only inert cosolute. Hence, for the present case, m_c is the molality of conjugate acid m_a. If Eqn. (1), describing the kinetics of reaction without inhibition, is substituted into Eqn. (4), describing the inhibiting effect of added unreactive cosolutes, we obtain

$$k(m_a, m_b) = [k(m_c = 0) + m_b k_b]e^{m_a a} \quad (5)$$

where a is the (rate-retarding) effect of the acidic form of the cosolute on both the water-catalysed and general-base catalysed reaction. The kinetic data were fitted to Eqn. (5). The results together with pK_a values are summarised in Table 1.

Fitting data to an equation related to Eqn. (5), in which the acidic form is assumed to inhibit only the water-catalysed reaction, leads to less satisfactory fits. Introducing an additional parameter to distinguish between rate-retarding effects on the water-catalysed and on the base-catalysed hydrolysis seems unjustifiable.

Employing the theory developed in previous work, the effect of the formation of encounter complexes from reactant(s) and an inert cosolute can be expressed in terms of G(c) values (for a brief review, see Ref. 11). These G(c) parameters were calculated using Eqn. (6) [cf. Eqns (2) and (3)]:

$$a = \frac{2}{RTm_0^2}G(c) - N\phi M_w \quad (6)$$

where m_0 is 1 mol kg^{-1}, N is the number of water molecules incorporated in the activated complex, ϕ is the

Table 1. General-base catalysed hydrolysis of **1a** at 298.2 K[a,c]

Parameter	Ethanoate	Chloroethanoate	Butanoate
pK_a[b]	4.76[9]	2.86[10]	4.82[9]
k_b (10^{-4} s^{-1} mol^{-1} kg)	16.63 (0.47)	2.08 (0.09)	20.61 (0.22)
a (kg mol^{-1})	−0.30 (0.03)	−0.11 (0.02)	−0.56 (0.01)
$k(m_c = 0)$ (10^{-4} s^{-1})	13.2 (0.2)	13.0 (0.1)	13.0 (0.1)

[a] Numbers in parentheses are standard errors based on a least-squares fit of kinetic data using Eqn. (5).
[b] All pK_a values, except those for the α-amino acids and hydroxylamine, were obtained from a literature search using the Beilstein Crossfire system. Available values were collected, assessed and averaged. Only one, representative, reference is given for every pK_a.
[c] Second-order and third-order rate constants are given with units s^{-1} mol^{-1} kg and s^{-1} mol^{-2} kg^2, respectively. These unconventional units facilitate comparison with and introduction of G(c) values. The exceptions are rate constants for hydroxide-catalysed hydrolysis, given in s^{-1} mol^{-1} dm^3, as hydroxide concentrations were calculated from the pH of the solutions.

practical osmotic coefficient of water and M_w is the molar mass of water. N was set to 2, despite the fact that only one water molecule is involved in the rate-determining step for the general-base catalysed reaction. The error introduced in this way is small as the term $N\phi M_w$ is small and the contribution of general-base catalysis to the overall rate constant is generally less than 50%. The $G(c)$ values for ethanoate and butanoate of -327 ± 37 and -649 ± 13 J kg mol^{-2}, respectively, correspond to the corresponding values for the water-catalysed reaction. This correspondence supports the hypothesis that rate-retarding effects are similar for water-catalysed and for general-base catalysed hydrolysis and is consistent with the notion that the rate-retarding effects are (largely) caused by blocking of the reaction centre.

Using the results given in Table 1, a Brønsted plot was constructed for carboxylate general bases and water [Fig. 2, Eqn. (7)].

$$\log k_b = 0.29 pK_a - 4.24 \quad (7)$$

The Brønsted β is 0.29 ± 0.05, slightly lower than the value for activated amides without the phenyl substituent in the triazole ring.[1,12,13]

Interestingly, in terms of general-base catalysis, comparison of ethanoate and butanoate shows that the latter is slightly more effective. The observed difference cannot be explained on the basis of its slightly higher pK_a. This pattern could be caused by the more hydrophobic nature of butanoate, resulting in additional hydrophobic stabilisation of the encounter complexes formed between **1a** and butanoate in the initial stages of the activation process.

From base-catalysed hydrolysis to nucleophilic substitution

Phenylalaninamide hydrochloride and alaninamide hydrochloride are both rate retarding in their fully protonated form; $G(c) = -1869$ and -234 J kg mol^{-2}, respectively.[5,14] In their deprotonated forms, they can function as general bases. It was expected that especially phenylalaninamide, being strongly rate retarding in the protonated state and hence forming rather stable encounter complexes, would be an effective catalyst for hydrolysis.

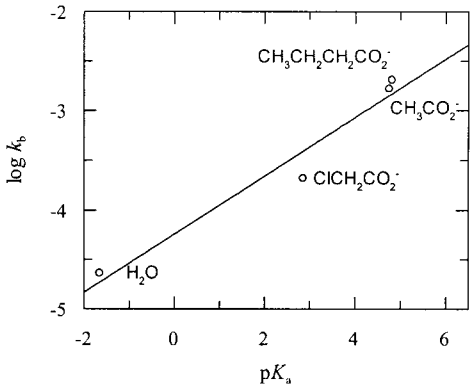


Figure 2. Brønsted plot for general-base catalysed hydrolysis of **1a**

Indeed, the reactivity of **1a** in the presence of unprotonated alaninamide and phenylalaninamide is high. The pH dependence of the reactivity of **1a** in the presence of alaninamide and phenylalaninamide (Fig. 3) indicates that high reactivity is indeed associated with deprotonation of the general base.

The results for alaninamide and phenylalaninamide were fitted to Eqn. (8), using a non-linear least-squares procedure:

$$k(m_c, \mathrm{pH}) = k(m_c) + k_{OH} \times 10^{\mathrm{pH}-14} + \frac{k_{\mathbf{1a}} m_c}{1 + 10^{pK_a - \mathrm{pH}}} \quad (8)$$

The first term on the right-hand side, $k(m_c)$, is the rate

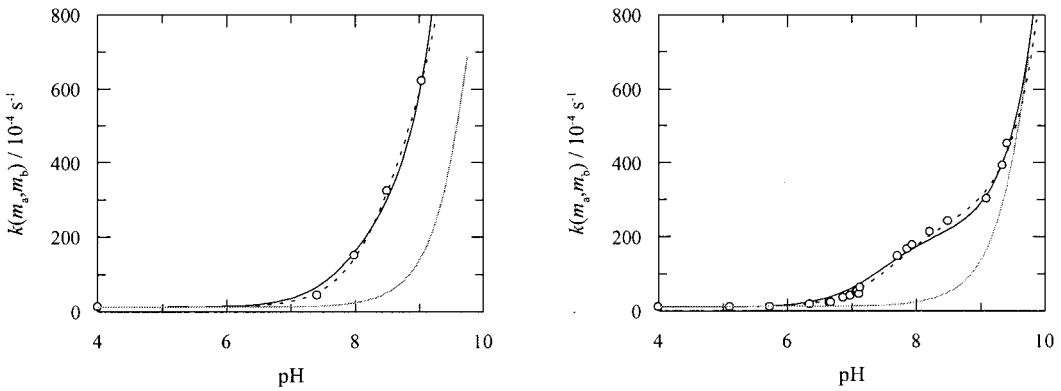


Figure 3. pH dependence of the reactivity of **1a** in the presence of α-amino acid derivatives. Left: 0.11 mol kg^{-1} alaninamide with pK_a set to 8.02 (solid line) and pK_a 8.58 (dotted line). Right: 0.11 mol kg^{-1} phenylalaninamide, pK_a set to 7.45 (solid line) and pK_a 7.69 (dotted line). Grey lines indicate hydroxide-catalysed hydrolysis

Table 2. Reactivity of **1a** in the presence of alaninamide and phenylalaninamide at 298.2 K[a]

Parameter	Alaninamide	Phenylalaninamide
pK_a	8.58 (lit.[15]: 8.02)	7.69 (lit.[15]: 7.45)
$k_{\mathbf{1a}}$ (s^{-1} mol^{-1} kg)	0.51 (0.17)	0.22 (0.02)
$k(m_c)$ (10^{-4} s^{-1})	12.3	10.3
k_{OH} (10^2 s^{-1} mol^{-1} dm^3)	n.s.[b]	7.3 (0.8)

[a] Numbers in parentheses are standard errors based on a least-squares fit of kinetic data using Eqn. (8).
[b] The value of 1720 (930) s^{-1} mol^{-1} dm^3 obtained from the curve-fitting procedure cannot be regarded as significant considering the error margin.

constant for hydrolysis at pH 4 in the presence of m_c molal protonated cosolute c. The second term on the right-hand side yields the rate of hydroxide-ion catalysed hydrolysis with k_{OH} the second-order rate constant for hydroxide-catalysed hydrolysis and 10^{pH-14} the concentration of hydroxide. The third term on the right-hand side represents the rate of reaction with the cosolute, where k_{1a} is the rate constant of reaction of the cosolute with **1a**, m_c is the total molality of cosolute and $(1 + 10^{pK_a - pH})^{-1}$ is the fraction of cosolute in the deprotonated form. The results are summarised in Table 2.

The rate constants, $51 \pm 17 \times 10^{-2}$ and $22 \pm 2 \times 10^{-2}$ s^{-1} mol^{-1} kg for alaninamide and phenylalaninamide, respectively, are significantly higher than the values of 12.2×10^{-3} and 8.33×10^{-3} s^{-1} mol^{-1} kg predicted on the basis of the Brønsted plot for general-base catalysed hydrolysis and their literature pK_as of 8.02 and 7.45, respectively[15] [the pK_a values obtained from fitting to Eqn. (8) lead to only marginally higher predicted values]. We attribute this marked difference in reactivity of **1a** for these general bases to a change in reactivity from general-base catalysed hydrolysis to nucleophilic substitution. The UV/Vis properties of amides, the expected products of nucleophilic substitution reactions, are only slightly different from those of the products of hydrolysis. Hence the spectral changes upon reaction (followed at a single wavelength) give no information about the actual reaction occurring, other than that the amide functionality is reacting. However, comparison with the literature[16,17] reveals that the difference spectrum upon reaction is in accord with formation of an amide instead of a carboxylate. The structurally related activated amide 1-acetyl-1,2,4-triazole has also been shown to undergo general-base catalysed hydrolysis and nucleophilic substitution by a variety of nucleophiles.[18]

The rate constant for hydroxide-catalysed hydrolysis of **1a** in the presence of phenylalaninamide is considerably lower than the rate constant of 1130 s^{-1} mol^{-1} dm^3 for hydroxide-catalysed hydrolysis of **1a** without cosolute.[13] Previously, the effect of 2-methylpropan-2-ol on the hydroxide-ion catalysed hydrolysis of **1a** has been studied.[12] At low mole fractions of added 2-methylpropan-2-ol, the second-order rate constants (k_{OH}) showed a maximum when plotted against the mole fraction of 2-methylpropan-2-ol. This maximum was attributed to a destabilisation of the initial state of the hydroxide-ion catalysed hydrolysis. This destabilisation of the initial state has been shown to originate from the unfavourable Gibbs energy of transfer of the hydroxide anion from water to water with cosolute, more than cancelling the corresponding favourable Gibbs energy of transfer of **1a**. For neutral hydrolysis of **1a** in aqueous solutions containing 2-methylpropan-2-ol,[19] $G(c) = -392$ J kg mol^{-2}. $G(c)$ for the same reaction in the presence of phenylalaninamide hydrochloride[14] is -1869 J kg mol^{-2}. Compared with solutions with added 2-methylpropan-2-ol, this pattern indicates that the standard chemical potential of **1a** is lowered more in solutions with added phenylalaninamide. Therefore, added phenylalaninamide could lead to a rate decrease if the stabilising effect of phenylalaninamide more than cancels the expected destabilising effect of phenylalaninamide on the hydroxide anion. However, the effect of phenylalaninamide on the standard chemical potential of hydroxide anion is unknown. Hence, reliable estimates of k_{OH} cannot be made. Related to the unknown effect of the added cosolute on the standard chemical potential of the hydroxide anion, the effect of added cosolute on the water self-ionization constant is unknown. Hence, using $[OH^-]$ based on the observed pH should be done with caution.

The values for pK_a obtained using Eqn. (8) as given in Table 2 are both higher than literature values. Together with the observed pattern in the deviation between the experimental and calculated values (Fig. 3, right-hand side), this trend indicates that Eqn. (8) underestimates the rate constants at higher pH. As nucleophilic substitution can be general-base catalysed, the increasing molalities of unprotonated amine not only increase nucleophile molalities, but also increase catalyst molalities. In addition, at lower pH, the rate constants might be underestimated as a result of inhibition of reaction by the protonated amine. In order to test the hypothesis of general-base catalysed nucleophilic substitution, the effect of both ethanoate/ethanoic acid and butanoate/butanoic acid buffers on the rate of reaction of **1a** with phenylalaninamide was determined (Fig. 4).

According to the plot on the left-hand side of Fig. 4, the rate of reaction between **1a** and alaninamide increases

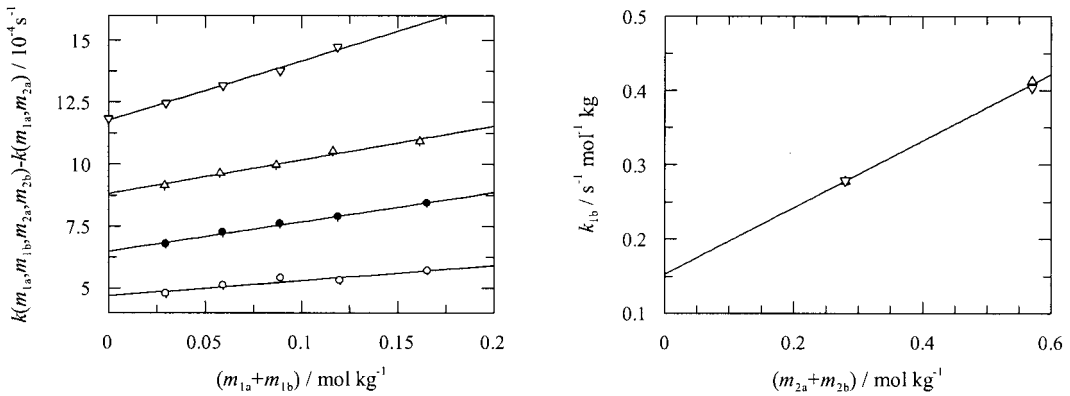


Figure 4. Reactivity of **1a** with alaninamide and phenylalaninamide in the presence of ethanoate/ethanoic acid and butanoate/butanoic acid buffers. Left: the contributions of general-base catalysed hydrolysis and nucleophilic substitution to the observed rates as a function of total molality of alaninamide hydrochloride and alaninamide ($m_{1a} + m_{1b}$) at total concentrations of ethanoate/ethanoic acid (3:1) buffers ($m_{2b} + m_{2a}$) of (○) 0.27, (●) 0.44, (△) 0.56 and (▽) 0.75 mol kg^{-1}. Right: Second-order rate constant k_{1b} for nucleophilic substitution by phenylalaninamide as a function of total molality ($m_{2a} + m_{2b}$) of (△) ethanoate/ethanoic acid and (▽) butanoate/butanoic acid molality

with increasing buffer concentration. The contribution of general-base catalysed hydrolysis and (general-base catalysed) nucleophilic substitution is calculated from the observed rate constant $k(m_{1a},m_{1b},m_{2a},m_{2b})$ and the calculated rate constant of the water-catalysed reaction in the presence of only the rate retarding protonated cosolutes $k(m_{1a},m_{2a})$. The values obtained in this way were not corrected for rate-retarding effects. A similar pattern is found for the reaction of phenylalaninamide with **1a**. This pattern indicates that general-base catalysed nucleophilic substitution is indeed occurring, which explains the deviating pK_a values from the curve fits. From a plot of the corresponding second-order rate constants as a function of total buffer molality $m_{2a} + m_{2b}$ (Fig. 4, right-hand side), the rate constant for uncatalysed nucleophilic substitution k_{nuc} can be determined. For phenylalaninamide, a value of 0.153 ± 0.007 s^{-1}mol^{-1}kg is obtained (cf. Table 2). Unfortunately, based on the available data, a value for k_{nuc} for alaninamide cannot be determined.

Using hydroxylamine, an even higher reactivity than that observed for alaninamide and phenylalaninamide was found, despite its lower pK_a (Fig. 5).

The observed rate constant of 53.1 ± 7.4 s^{-1} mol^{-1} kg (104.9 ± 14.8 s^{-1} mol^{-1} kg based on only deprotonated hydroxylamine, $k_{HONH_2.HCl} = 0.69 \pm 0.03$ s^{-1} mol^{-1} kg) is exceptionally high. Error margins are based on inclusion of the apparent outlier at 5.2 mmol kg^{-1} and the notion that a negative intercept is physically unrealistic. The negative intercept could be caused by two factors. First, a small change in protonation (the pH decreases slightly) upon dilution of the hydroxylamine buffer stock solution. Second, a second-order (in total hydroxylamine molality) term corresponding to general-base or general-acid catalysed nucleophilic substitution. Both effects would lead to an overestimate of the second-

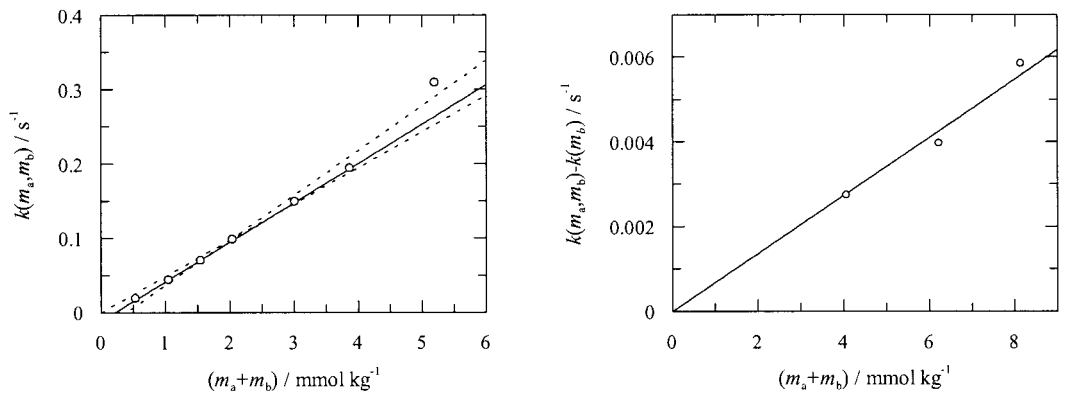


Figure 5. Reactivity of **1a** in the presence of hydroxylamine and hydroxylamine hydrochloride. Concentrations are total concentrations. Left: hydroxylamine and hydroxylamine hydrochloride in 1:1 ratio, pH = 5.91 ± 0.06. Dotted lines indicate error margins (see text). Right: hydroxylamine hydrochloride, pH = 3.76 ± 0.03

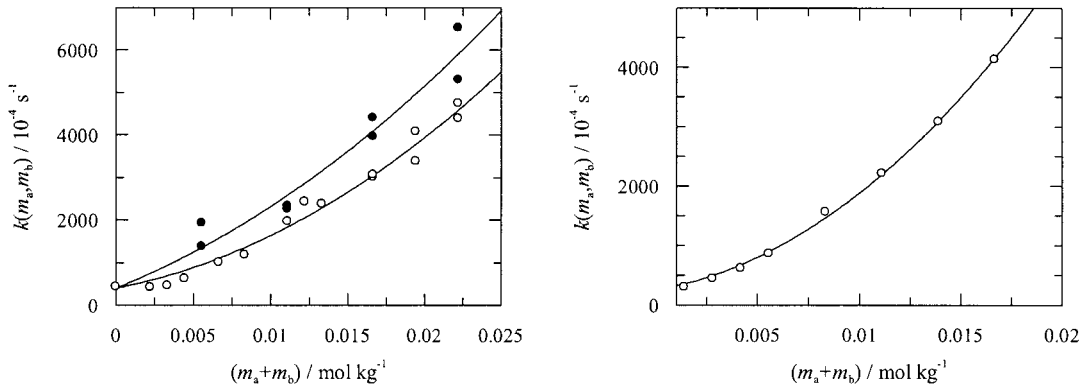


Figure 6. Observed rates of reaction for **1a**. Left: in the presence of (○) n-propylamine and (●) *n*-pentylamine. Buffer ratios are 1:9 (base:acid), pH = 9.54 ± 0.07 for n-propylamine and 9.53 ± 0.03 for n-pentylamine. Right: in the presence of benzylamine. Buffer ratio is 1:1 (base:acid) and pH = 9.34 ± 0.04

order rate constant of reaction. Based on the pK_a of 5.96[20] and Eqn. (7), the expected rate constant is 40.1×10^{-4} s^{-1} mol^{-1} kg for general-base catalysis. This large discrepancy between the predicted value for general-base catalysed hydrolysis and the observed value again strongly suggests that the reaction pathway that is followed is not general-base catalysed hydrolysis. The high reactivity of hydroxylamine is often accounted for in terms of the α-effect,[21] therefore the results are indicative for nucleophilic attack on **1a**. Hydroxylamine has two nucleophilic centres (of different reactivity), further enhancing reactivity. Nucleophilic attack by hydroxylamine will occur on the amide functionality of **1a**, eventually leading to *N*-hydroxybenzamide. Surprisingly, the hydroxyl moiety is most nucleophilic in hydroxylamine in the case when *p*-nitrophenyl acetate is the substrate undergoing nucleophilic attack, resulting in an initial excess of the product from nucleophilic attack by the hydroxyl moiety. However, this initial unstable product can react further to *N*-hydroxybenzamide.[22,23] The zwitterionic form of hydroxylamine is not present in detectable amounts,[20,23] practically excluding the *O*-deprotonated hydroxylamine as the nucleophile.

Using n-propyl- and n-pentylamine as well as benzylamine, again high reactivities were found but the observed rate constants were not linear with concentration of general base (Fig. 6). We attribute this pattern to general-base catalysed nucleophilic substitution,[4] but general-acid catalysis (by the conjugate acids) cannot be excluded.[3,24,25]

The observed rate constants were fitted to the equation

$$k(m_c) = k_{pH} + k_{2nd}m_c + k_{3rd}m_c^2 \qquad (9)$$

where k_{pH} is the (pseudo-)first-order rate constant for reaction in the absence of added general base at the experimental pH, k_{2nd} is the second-order rate constant based on total buffer concentration for nucleophilic substitution (and a minor fraction general-base catalysed hydrolysis) by cosolute c, m_c is the molality of cosolute c and k_{3rd} is the third-order rate constant for the general-base catalysed nucleophilic substitution. Assuming that general-base catalysed hydrolysis makes a negligible contribution to the observed rate, k_{nuc} can be calculated (Table 3) from k_{2nd}.

According to Table 3, n-pentylamine provides the most effective non-catalysed nucleophilic substitution. However, the increase in reactivity appears to be too large to be caused solely by the difference in pK_a and different steric effects. This increase in reactivity points towards more favourable interactions between n-pentylamine and **1a** compared with those between n-propylamine and **1a**.

Table 3. Nucleophilic substitution of **1a** in aqueous solution at 298.2 K in the presence of amine/ammonium HCl buffers[a,b]

Parameter	n-Propylamine	n-Pentylamine	Benzylamine
pK_a	10.66[26]	10.64[27]	9.36[28]
k_{2nd} (s^{-1} kg mol^{-1})	6.9 (1.8)	14.5 (4.6)	5.5 (0.8)
k_{nuc} (s^{-1} kg mol^{-1})[c]	69 (18)	145 (46)	11.1 (1.6)
k_{3rd} (10^2 s^{-1} kg^2 mol^{-2})	5.4 (1.0)	4.6 (2.4)	10.7 (0.6)

[a] The numbers in parentheses are standard errors based on a least-squares fit of the kinetic data using Eqn. (9).
[b] $k_{nuc} = 10k_{2nd}$, the rate effect of the protonated amine in this concentration range is expected to be negligible.[29]
[c] Solvent kinetic isotope effect of 1.6 was found for the reaction. However, many factors influence this isotope effect, including the shift in pK_a of the amine upon changing from H_2O to D_2O as the solvent.

The second-order terms (in amine), however, are comparable (although care has to be taken in interpreting the value for n-pentylamine) suggesting that self-association of n-pentylamine is comparable to self-association of n-propylamine. Previously, for nucleophilic substitution of sufficiently hydrophobic *p*-nitrophenyl esters by hydrophobic amines, the second-order (in nucleophile) process was found to be more effective because the reactants tend to cluster.[30,31]

The reactivity of benzylamine in comparison with that of the alkylamines is surprisingly high taking into account its lower pK_a. Given the present data set, the second-order term (in amine) cannot be compared with second-order terms for the alkylamines. The buffer ratio is different and the influence of basicity (or acidity) on general-base (or general-acid) catalysis of nucleophilic substitution is unknown, because Brønsted plots for general-base catalysis and general-acid catalysis of nucleophilic substitution have not been determined.[18]

An experiment, conducted under the conditions of the kinetic runs, was performed on a milligram (of substrate) scale. Benzylamine was used as nucleophile and **1b** instead of **1a** was used as substrate. Both ^{1}H NMR and ^{13}C NMR spectra of the product correspond to literature spectra of *N*-benzylbenzamide,[32–34] corroborating the view that nucleophilic substitution did indeed take place. Reaction between **1b** and phenylamine in benzene was shown to yield *N*-phenylbenzamide.[35] Moreover, **1b** has been used as a benzoylation reagent in dry cyanomethane,[36] both consistent with the possibility of nucleophilic substitution of **1b** and the related **1a**.

An overall Brønsted plot of rate constants for general-base catalysed hydrolysis k_b and uncatalysed nucleophilic substitution k_{nuc} of **1a** (both indicated by $k_{\mathbf{1a}}$) shows considerable scattering (Fig. 7). Note that in all LFERs, only the linear terms describing the bimolecular (uncatalysed) nucleophilic substitution reaction have been used.

For general bases with comparable nucleophilic groups, a correlation is obtained between observed rate constant and pK_a. However, the correlation does not extend over different groups of bases, in agreement with extensive literature data.[37,38] Remarkably, the observed rate constants for aromatic amines acting as a nucleophile seem to be consistently higher than those for non-aromatic amines with the same pK_a would have been, i.e. the data points for aromatic amines lie above and to the left of a line through the non-aromatic amines.

An interesting case is presented by aspartame (Asp-PheOMe) (Scheme 4). Aspartame offers a number of functional groups, the carboxylic acid and the amine being the most important for the present study. The carboxylate group is expected to be a general-base catalyst in the hydrolysis reaction of **1a**. The amine group will act as nucleophile, the relative importance of both reactions being dependent on the degree of protonation of

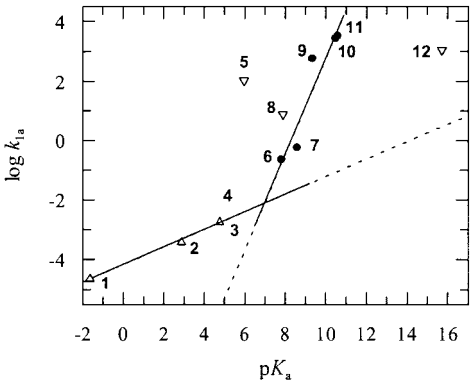
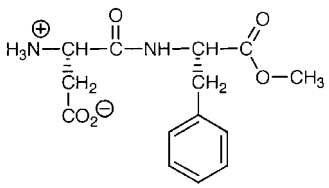


Figure 7. Plots of k_b and k_{nuc} (indicated by k_{1a}) vs pK_a for **1a**. (△) General-base catalysis by: water (1), chloroethanoate (2), ethanoate (3) and butanoate (4). (●) Nucleophilic substitution by amines, viz. phenylalaninamide (6), alaninamide (7), benzylamine (9), propylamine (10) and pentylamine (11). (▽) Nucleophilic substitution by other compounds, viz. hydroxylamine (5), aspartame (8) and hydroxide (12). The line through the data for amines was drawn to guide the eye

both groups. From the pK_a of the carboxylic acid functionality[39,40] of 3.2, we conclude that in the molality range up to 0.044 mol kg^{-1} of aspartame, the contribution of the carboxylate group to the observed rate of reaction is negligible. The pH–rate profile for reaction of **1a** with aspartame at three concentrations of aspartame is given in Fig. 8.

From the non-linear least-squares fits to the observed rate profiles, the second-order rate constant for nucleophilic substitution on **1a** by aspartame is 7.8 ± 0.2 s^{-1} mol^{-1} kg. As can be seen from Fig. 7, this rate constant for nucleophilic substitution is clearly much higher than expected. We attribute this increase to two factors. First, aspartame is a rather hydrophobic molecule, which could form hydrophobically stabilised encounter complexes (cf. the effect of benzylamine). Second, the nucleophilic substitution by aspartame can be intra-molecularly general-base catalysed by the carboxylate functionality, thereby greatly enhancing the nucleophilicity of aspartame (Scheme 5). It is interesting to view aspartame as a cyclised, non-nucleophilic general base catalyst for hydrolysis. The molecule consists of two α-amino acids, and has a hydrophobic 'binding site' and a 'catalytic center'.



Scheme 4

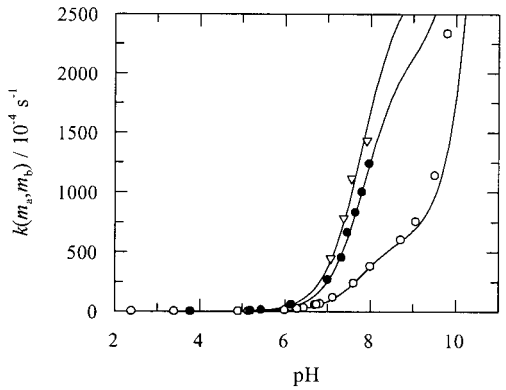


Figure 8. pH–rate profile for **1a** in the presence of different total molalities $m_a + m_b$ of aspartame: (○) 0.007; (●) 0.028; (▽) 0.044 mol kg^{-1}. The stability of aspartame under these conditions is reasonable in comparison with the reaction under study (see Ref. 40). The pH of aspartame solutions was adjusted (from a pH in the range 4–7) immediately before performing the measurement

General-base catalysed hydrolysis and uncatalysed nucleophilic substitution on 1a; a linear free energy relationship

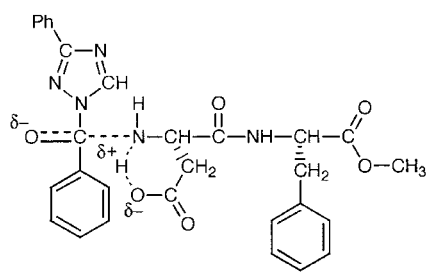
Despite the fact that comparing basicities of nucleophiles with kinetic nucleophilic reactivities towards carbon compounds is not a proper rate-equilibrium comparison (Parker[41] and Hine and Weimar[42] developed the concept of 'carbon basicity') reactivity of general bases in nucleophilic substitution is roughly correlated with their basicity. However, there are many factors influencing reactivity other than basicity,[43] the most important being the elusive α-effect[21] and steric factors. Often, nucleophilicity varies with basicity within a series of compounds, which can be attributed to differences in solvation of different nucleophilic groups and a difference in hardness/softness of the nucleophile. In view of the failure of basicity as an indicator of nucleophilicity, alternative scales of nucleophilicity have been developed, most importantly Richie's[44] N_+ (for nucleophilic addition on sp^2 carbon) and the Swain and Scott[45] n parameter (for nucleophilic substitution on sp^3 carbon). There are links between the two scales,[46] but a unifying scale for nucleophilicity does not exist.

We compare rate constants for uncatalysed nucleophilic substitution and general-base catalysed hydrolysis of **1a** with the rate constants for the same reactions of *p*-nitrophenyl acetate (*p*-NPA). There are ample experimental data on nucleophilic substitution on *p*-NPA[2,23,47–50] and the available data for *p*-NPA form a reliable basis for linear free energy relationships (LFERs).[51] Most importantly, however, the change in reaction pathway from general-base catalysed hydrolysis to nucleophilic substitution of **1a** is mirrored by *p*-NPA. Both are carbonyl compounds showing approximately the same reactions with added general bases. Also, the anions of both 3-phenyl-1,2,4-triazole and 4-nitrophenol are reasonably good leaving groups based on the pK_a values of the parent compounds of 9.58[52] and 7.15,[53] respectively.

When log k_{1a} is plotted as a function of log k_{p-NPA} for different bases/nucleophiles, a linear correlation is found (Fig. 9) with a slope of 0.91. The correlation includes data points for hydroxylamine (an α-effect nucleophile) and hydroxide anion (a charged nucleophile).

Interestingly, the LFER spans regions of different reactivity; general-base catalysed hydrolysis for both **1a** and *p*-NPA by water (1) and ethanoate (3)[54] and nucleophilic substitution by hydroxylamine (5), benzylamine (9), propylamine (10), pentylamine (11) and hydroxide (12). This pattern is in line with the argument formulated by Fersht and Jencks[55] that 'such a correlation shows only that the two compounds being compared have similar transition states for each individual reaction under consideration; if there is a change in the nature of the transition state with changing nucleophile … the correlation shows that this change takes place in a similar manner for both compounds.'

We conclude that the enhanced reactivity compared with what would be expected on the basis of general-base catalysis alone is indeed caused by a change in mech-



Scheme 5

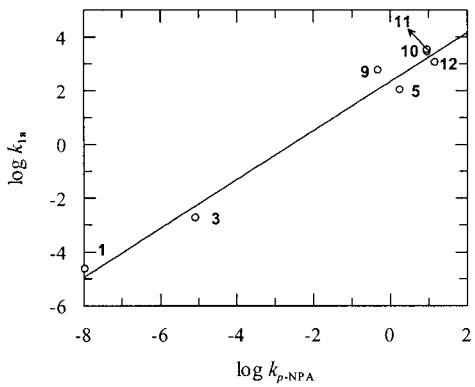


Figure 9. Linear free energy relationship between nucleophilic substitution on **1a** and nucleophilic substitution on *p*-NPA by different bases/nucleophiles. Numbering as in Fig. 7

anism to nucleophilic substitution. The linear free energy relationship also allows reliable estimates to be made of the rates of uncatalysed nucleophilic substitution on **1a**.

General-base catalysis, nucleophilic substitution and inhibition

As described above, a range of possible interactions between cosolute and hydrolytic probe sometimes result in rather complex reactivity patterns. For example, phenylalaninamide and alaninamide show rate-decreasing effects in their protonated forms, but are strong nucleophiles in their deprotonated forms. Similarly, the effect of the carboxylate buffers is a composite effect of the rate retardation by the protonated form and catalysis by the carboxylate.

Previously,[56] rate effects induced by α-amino acids have been studied at a pH of 4.0, at which the α-amino acids are present mainly in their zwitterionic forms. Rate-enhancing effects were found for a range of α-amino acids and even for some of their derivatives. Rate-enhancing effects were not found to correlate with the pK_a of the carboxylic acid moiety. Furthermore, the kinetic solvent isotope effect did not change significantly and no linearity of the slope of k_{obsd} versus molality of cosolute was observed. Hence, the kinetic effects were 'not governed by general-base catalysis of the α-amino acid carboxylate group, but involve medium effects instead.'[56] The kinetic analyses presented here, however, indicate that apart from general-base catalysis by the α-amino acid carboxylate group, nucleophilic substitution by the α-amino acid amine group is also a possible reaction pathway. Consequently, the observed rate effects are rather difficult to interpret, as a linear Brønsted plot is not to be expected. In addition, given the observed G(c) values and the data in Fig. 4.1 of Ref. 56, it is difficult to draw conclusions about the linearity of the observed rate effects with molality of cosolute. This is especially the case since possible general-base or general-acid catalysis will produce deviations from linearity in plots of $k(m_c)$ versus molality. The deviation is towards higher $k(m_c)$ at higher molalities, which can accidentally produce good linear plots of $\ln[k(m_c)/k(m_c = 0)]$ against cosolute molality m_c. Finally, the observed kinetic solvent isotope effect of 2.49 for the hydrolysis of **1a** in 0.5 mol kg^{-1} glycine at pH 4 is very similar to the 2.69 observed for the reaction in water at pH 4 without cosolute. Unfortunately, however, this value will be influenced by the increase in pK_a of both the α-amino acid carboxylate and the α-amino acid amine functionality upon changing the solvent from H_2O to D_2O. The increase in pK_a for the glycine carboxylate group[57] of 0.39 results in a larger fraction of the α-amino acid carboxylate group becoming neutralised. Together with the expected kinetic solvent isotope effect, this leads to a decrease in rate constant. Consequently, the observed rate of the nucleophilic substitution reaction will be decreased, even though no proton transfer takes place in the uncatalysed nucleophilic substitution reaction. The increase in pK_a of the α-amino acid amine functionality[57] of 0.63 results in fewer free amine groups being available for reaction, also leading to a decrease in observed rate constant. Hence the similarity in kinetic solvent isotope effects could be merely coincidental, which interfered with the interpretation of the result obtained for α-amino acids.

We contend that for the least hydrophobic α-amino acids, rate-retarding effects are negligible and only rate enhancements are observed caused by general-base catalysed hydrolysis and nucleophilic substitution.[56] The effect of general-base catalysis of hydrolysis can be calculated using Eqn. (7), pK_a values of the α-amino acids[58] and pH (Table 4).

If the residual observed rate enhancements are attributed to uncatalysed nucleophilic substitution by free amine, excellent correlation is again observed (Fig. 10) with data for *p*-NPA.

Hence, even though the fraction of unprotonated amine functionalities is of the order of ppm, the unprotonated amine functionality of α-amino acids is strongly nucleophilic, rendering nucleophilic substitution kinetically detectable at a pH as low as 4.0 for hydrophilic α-amino acids. More hydrophobic α-amino acids, however, show rate retardation as the main effect at a pH of 4.0. However, the observed rate retardations will be a combined effect of inhibition by the hydrophobic α-amino acid, general-base catalysis and nucleophilic substitution.

Table 4. Reactivity of **1a** in aqueous solution at 298.2 K in the presence of α-amino acids[a]

α-Amino acid	pK_a[b]	G(c)	$k(m_c = m_0)$ (10^{-4} s^{-1})	$m_b k_b$[c] (10^{-4} s^{-1})	k_{nuc} (10^2 kg mol^{-1} s^{-1})
Glycine	2.35, 9.78	875 (21)	24.5 (0.5)	2.8 (1.3)	5.8 (1.5)
Alanine	2.34, 9.69	558 (16)	19.0 (0.3)	2.7 (1.2)	2.0 (0.8)
Valine	2.32, 9.62	467 (9)	17.6 (0.2)	2.7 (1.2)	1.2 (0.6)
Leucine	2.36, 9.60	518 (21)	18.4 (0.4)	2.8 (1.3)	1.4 (0.6)

[a] Numbers in parentheses are standard errors.
[b] In the calculation of the errors in G(c), $m_b k_b$ and k_{nuc}, the errors in the pK_a values were set to 0.05 and 0.1 for the first and second pK_a, respectively.
[c] Calculated using Eqn. (7).

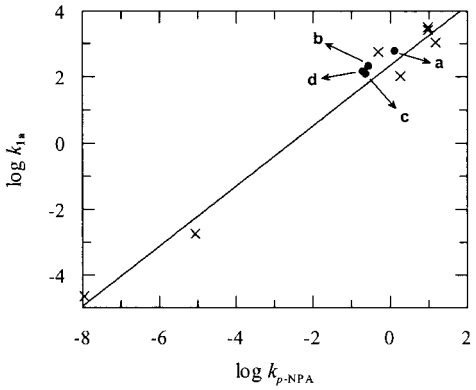


Figure 10. (●) Data for nucleophilic substitution on **1a** by α-amino acids, glycine (a), alanine (b), valine (c) and leucine (d), compared with (X) the LFER as described in Fig. 9. Error margins in log k_{1a} are 0.6, 0.9, 1.1 and 1.0 for glycine, alanine, valine and leucine, respectively

CONCLUSIONS

In aqueous solutions containing general bases, activated amide **1a** is subject to water-catalysed hydrolysis, general-base catalysed hydrolysis with a Brønsted β of 0.29 and nucleophilic substitution with a Brønsted β of $\sim$1.5 for amine nucleophiles. In certain cases, nucleophilic substitution is general-base and/or general-acid catalysed. Reactivities of more hydrophobic general bases seem to be consistently higher than reactivities of hydrophilic general bases supporting an explanation based on the formation of hydrophobically stabilised encounter complexes. In future studies, rate effects of cosolutes, in particular rate-enhancing effects, should be scrutinised for unexpected catalytic effects or changes in mechanism. In the present study we have shown that small fractions of compounds present in, e.g., a deprotonated state, albeit in ppm, can induce large rate effects.

EXPERIMENTAL

Materials. All buffers were made from commercially available acids, alkylamines or benzylamine (from Acros or Aldrich) using aqueous NaOH or aqueous HCl of known concentration (Titrisol). Aspartame was kindly provided by Professor Dr H. E. Schoemaker (DSM/University of Amsterdam). 1-Benzoyl-3-phenyl-1,2,4-triazole (**1a**) and 1-benzoyl-1,2,4-triazole were synthesised according to literature procedures.[13,59,60]

Kinetic Experiments. Aqueous solutions were prepared by weight immediately before use. Buffers were prepared by partially (to the desired buffer ratio) neutralising the corresponding acid by adding the appropriate amount of 1.000 mol l^{-1} aqueous NaOH by volume or by weight. Buffer ratios were routinely accurate to within 1%. Buffer solutions containing n-propylamine and n-pentylamine were prepared by addition of the appropriate amount of 1.000 M aqueous HCl within 2 min prior to monitoring the reaction of **1a** in order to prevent evaporation of the volatile amines from the solutions. Water was distilled twice in an all-quartz distillation unit. All reactions were monitored at 273 nm (or the lowest possible wavelength above 273 nm if a given cosolute had absorption bands at that wavelength) and at 25.0 $\pm$ 0.1 °C. Amide **1a** was injected as 5–7 μl of a stock solution containing **1a** in cyanomethane into about 2.8 ml of an aqueous solution of cosolute in a concentration range in which the reaction could be followed in a stoppered 1.000 cm quartz cuvette. The resulting concentrations were about 10^{-5} mol dm^{-3} or less. The pH of all solutions was checked at the end of each kinetic experiment using either a Ross semi-micro combination pH electrode or a Sentron ISFET pH probe and was found to correspond well (not more than 0.2 pK_a units below) with the predicted pH from the pK_a and the buffer ratio. NMR spectra were recorded on Varian Gemini 200 (^{1}H: 200 MHz) and VXR 300 (^{1}H: 300 MHz) spectrometers.

Acknowledgement

Dr Matt Fielden is thanked for helpful mechanistic discussions.

REFERENCES

1. Karzijn W, Engberts JBFN. *Tetrahedron Lett.* 1978; 1787–1790.
2. Johnson SL. *Adv. Phys. Org. Chem.* 1967; **5**: 237–330.
3. Bruice TC, Donzel A, Huffman RW, Butler AR. *J. Am. Chem. Soc.* 1967; **89**: 2106–2121.
4. Bruice TC, Hegarty AF, Felton SM, Donzel A, Kundu NG. *J. Am. Chem. Soc.* 1970; **92**: 1370–1378.
5. Streefland L, Blandamer MJ, Engberts JBFN. *J. Am. Chem. Soc.* 1996; **118**: 9539–9544.
6. Buurma NJ, Pastorello L, Blandamer MJ, Engberts JBFN. *J. Am. Chem. Soc.* 2001; **123**: 11848–11853.
7. Rispens T, Lensink MF, Berendsen HJC, Engberts JBFN. submitted for publication.
8. Lensink MF, Mavri J, Berendsen HJC. *J. Comput. Chem.* 1999; **20**: 886–895.
9. Headley AD, Starnes SD, Wilson LY, Famini GR. *J. Org. Chem.* 1994; **59**: 8040–8046.
10. Niazi MSK. *J. Chem. Eng. Data* 1993; **38**: 527–530.
11. Engberts JBFN, Blandamer MJ. *J. Phys. Org. Chem.* 1998, **11**: 841–846.
12. Karzijn W, Engberts JBFN. *Recl. Trav. Chim. Pays-Bas* 1983; **102**: 513–515.
13. Karzijn W. PhD Thesis, Rijksuniversiteit Groningen, 1979.
14. Streefland L, Blandamer MJ, Engberts JBFN. *J. Phys. Chem.* 1995; **99**: 5769–5771.
15. Chambers RW, Carpenter FH. *J. Am. Chem. Soc.* 1955; **77**: 1522–1526.
16. Ley H, Specker H. *Chem. Ber.* 1939; **72**: 192–202.
17. Moser CM, Kohlenberg AI. *J. Chem. Soc.* 1951; 804–809.
18. Fox JP, Jencks WP. *J. Am. Chem. Soc.* 1974; **96**: 1436–1449.
19. Blokzijl W, Engberts JBFN, Blandamer MJ. *J. Am. Chem. Soc.* 1990; **112**: 1197–1201.
20. Kallies B, Mitzner R. *J. Phys. Chem. B* 1997; **101**: 2959–2967.
21. Edwards JO, Pearson RG. *J. Am. Chem. Soc.* 1961; **84**: 16–24.

22. Jencks WP. *J. Am. Chem. Soc.* 1958; **80**: 4581–4584 and 4585–4588.
23. Jencks WP, Carriuolo J. *J. Am. Chem. Soc.* 1960; **82**: 1778–1786.
24. Jencks WP, Carriuolo J. *J. Am. Chem. Soc.* 1960; **82**: 675–681.
25. Bruice TC, Mayahi MF. *J. Am. Chem. Soc.* 1960; **82**: 3067–3071.
26. Lin B, Islam N, Friedman S, Yagi H, Jerina DM, Whalen DL. *J. Am. Chem. Soc.* 1998; **120**: 4327–4333.
27. Hoerr CW, McCorkle MR, Ralston AW. *J. Am. Chem. Soc.* 1943; **65**: 328–329.
28. Arrowsmith CH, Guo HX, Kresge AJ. *J. Am. Chem. Soc.* 1994; **116**: 8890–8894.
29. Hol P, Streefland L, Blandamer MJ, Engberts JBFN. *J. Chem. Soc., Perkin Trans. 2* 1997; 485–488.
30. Oakenfull D. *J. Chem. Soc., Perkin Trans. 2* 1973; 1006–1012.
31. Oakenfull D. *J. Chem. Soc., Chem. Commun.* 1970; 1655–1656.
32. Keck GE, Wager TT, McHardy SF. *Tetrahedron* 1999; **55**: 11755–11772.
33. Darbeau RW, White EH. *J. Org. Chem.* 1997; **62**: 8091–8094.
34. Knowles HS, Parsons AF, Pettifer RM, Rickling S. *Tetrahedron* 2000; **56**: 979–988.
35. Purygin PP, Pankov SV. *Russ. J. Org. Chem.* 1996; **32**: 871–873.
36. Humpf HU, Berova N, Nakanishi K, Jarstfer MB, Poulter CD. *J. Org. Chem.* 1995; **60**: 3539–3542.
37. Heo CKM, Bunting JW. *J. Chem. Soc., Perkin Trans. 2* 1994; 2279–2290.
38. Bruice TC, Lapinski R. *J. Am. Chem. Soc.* 1958; **80**: 2265–2267.
39. Maheswaran MM, Divakar S. *Indian J. Chem., Sect. A* 1991; **30**: 30–34.
40. Skwierczynski RD, Connors KA. *Pharm. Res.* 1993; **10**: 1174–1180.
41. Parker AJ. *Proc. Chem. Soc.* 1961; 371.
42. Hine J, Weimar Jr RD. *J. Am. Chem. Soc.* 1965; **87**: 3387–3396.
43. Bunnett JF. *Annu. Rev. Phys. Chem.* 1963; **14**: 271–290.
44. Ritchie CD. *Can. J. Chem.* 1986; **64**: 2239–2250.
45. Swain CG, Scott CB. *J. Am. Chem. Soc.* 1953; **75**: 141–147.
46. Bunting JW, Mason JM, Heo CKM. *J. Chem. Soc., Perkin Trans. 2* 1994; 2291–2300.
47. Um IH, Lee GJ, Yoon HW, Kwon DS. *Tetrahedron Lett.* 1992; **33**: 2023–2026.
48. Acher F, Wakselman M. *J. Org. Chem.* 1984; **49**: 4133–4138.
49. Jencks WP, Gilchrist M. *J. Am. Chem. Soc.* 1968; **90**: 2622–2637.
50. Tee OS, Gadosy TA, Giorgi JB. *Can. J. Chem.* 1997; **75**: 83–91.
51. Gregory MJ, Bruice TC. *J. Am. Chem. Soc.* 1967; **89**: 2121–2125.
52. Kröger C-F, Freiberg W. *Z. Chem.* 1965; **5**: 381–382.
53. Mock WL, Morsch LA. *Tetrahedron* 2001; **57**: 2957–2964.
54. Butler AR, Gold V. *J. Chem. Soc.* 1962; 1334–1339.
55. Fersht AR, Jencks WP. *J. Am. Chem. Soc.* 1970; **92**: 5442–5452.
56. Streefland L. PhD Thesis, University of Groningen, 2000; available online at http://www.ub.rug.nl/eldoc/dis/science/e.streefland/.
57. Li NC, Tang P, Mathur R. *J. Phys. Chem.* 1961; **65**: 1074–1076.
58. Fasman GD (ed). *Handbook of Biochemistry and Molecular Biology, Proteins* (3rd edn). CRC Press: Cleveland, OH, 1976.
59. Staab HA, Lüking M, Dürr FH. *Chem. Ber.* 1962; **95**: 1275–1283.
60. Mooij HJ, Engberts JBFN, Charton M. *Recl. Trav. Chim. Pays-Bas* 1988; **107**: 185–189.

